

PRIMER NOTE

Microsatellite markers for *Fragaria* from 'Strawberry Festival' expressed sequence tags

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Abstract

We present 37 microsatellite primer pairs developed from a cDNA library of *Fragaria ×ananassa* Duch. cv. Strawberry Festival. Polymorphism was high and the number of presumptive alleles of 13 expressed sequence tag–simple sequence repeats (EST–SSRs) in 70 strawberry cultivars ranged from five to 32 per primer pairs, averaging 16.1. Cross-species amplification was also high and ranged from 89% in *Fragaria vesca* L. to 100% in the progenitor species of octoploid strawberry, *Fragaria chiloensis* (L.) Duch. and *Fragaria virginiana* Duch.

Keywords: EST, *Fragaria*, microsatellite, PCR, SSR, strawberry

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The cultivated strawberry, *Fragaria ×ananassa* ($2n = 8x = 56$), is an economically important fruit crop belonging to the Rosaceae family. The genus *Fragaria* consists of approximately 20 wild species with a basic chromosome number $x = 7$ and exists at four ploidy levels: diploid, tetraploid, hexaploid and octoploid (Hancock 1999). The cultivated strawberry has an allooctoploid genetic background originating from an accidental cross between a white-fruited *Fragaria chiloensis* brought to France from Chile in 1714 and a red-fruited *Fragaria virginiana* species brought from the Virginia colony to Europe in the 1600s. At least two major diploid ancestors are thought to have contributed to the domestic octoploid strawberry gene pool (Bringhurst 1990). Strawberry plants are vegetatively propagated and can be easily misidentified based on phenotype. Our objective was to develop simple sequence repeat (SSR) markers from a 'Strawberry Festival' expressed sequence tag (EST) library for use in identifying strawberry germplasm held at the US Department of Agriculture, Agricultural Research Service, National Clonal Germplasm Repository in Corvallis, Oregon.

SSR primer pairs were derived from an EST library constructed in Lambda ZAP vectors (Stratagene) from

salicylate-treated mixed vegetative tissues of *F. ×ananassa* cv. Strawberry Festival (Folta *et al.* 2005). The SSRIT software (Temnykh *et al.* 2001) was used to detect microsatellite-containing ESTs. From a collection of 1847 ESTs, 165 unique SSR-containing sequences were identified which contained an SSR motif of at least $(XX)_5$ or $(XXX)_4$. Primer pairs were successfully designed for 46 of 50 unique sequences using PRIMER 3 (Rozen & Skaletsky 2000). The design criteria included a polymerase chain reaction (PCR) product length between 100 and 350 bp in length, a T_a of 57–64 °C (optimum 60 °C), and a primer length of 20–25 bp (optimum 22 bp). Primer pairs were denoted University of Florida *F. ×ananassa* followed by a five-character alphanumeric EST code (i.e. UFFa 00 X 00) (Table 1).

Each primer pair was then used to amplify DNA from a subset of 12 strawberry clones, which contained two cultivars of diploid *Fragaria vesca* ('Baron Solemacher' and 'Yellow Wonder') and 10 octoploid genotypes. The octoploids consisted of six cultivars of *F. ×ananassa* ('Earliglow', 'Florida 90', 'Totem', 'Sweet Charlie' and parents of an octoploid mapping population 'Delmarvel' and 'Selva'), one wild clone of *F. chiloensis* (PI 551445), and three of *F. virginiana* (PI 551471, PI 612486, and PI 612491). PCRs were performed in 10 µL volume containing 1× reaction buffer, 2 mM MgCl₂, 0.2 mM dNTPs, 0.3 µM of each primer, 0.25 U of Biolase Taq DNA polymerase (Bioline), and 2.5 ng genomic DNA. The optimum annealing temperature (T_a) for a primer

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Table 1 Thirty-seven EST-SSR forward (F) and reverse (R) primer pairs, GenBank Accession nos, motifs, optimum annealing temperatures (T_a), expected size of the PCR product in bp, and amplification results from one clone of *Fragaria chiloensis*, and amplification and polymorphism results from six cultivars of *Fragaria ×ananassa*, two cultivars of *Fragaria vesca* and three clones of *Fragaria virginiana*

Locus	GenBank Accession no.	Primer sequences	Motif	T_a	Expected size	Amplification and polymorphism			
						<i>F. ×ananassa</i>	<i>F. vesca</i>	<i>F. chiloensis</i>	<i>F. virginiana</i>
UFFa 01D03	CO816689	F-TTACTGAAATGGGTTTCAGAGC R-GACAGCACAGTCATGGAAGATG	(TCT) ₅	61	236	+/-	+/-	+	+/-
UFFa 01E03*	AJ870458	F-ACCCCATCTTCTTCAAATCTCA R-GACAAGGCCAGAGCTAGAGAAG	(CAC) ₁₀	59	185	+/+	+/-	+	+/-
UFFa 01H05*	AJ870459	F-GGGAGCTTGCTAGCTAGATTG R-AGATCCAAGTGTGGAAGATGCT	(CT) ₈	64	246	+/+	+/-	+	+/+
UFFa 02A03	CO816743	F-GAGCTACACAAATGCCATCAAAA R-GCGCATTGCACTCTGTAACCTCT	(AG) ₁₂	50	168	+/+	+/-	+	+/+
UFFa 02C07	CO816760	F-CTCTCCCCACAAAACCCTAAAC R-AAAGATCGGTAGGCACAGAGAG	(CT) ₂₀	66	167	+/+	+/-	+	+/+
UFFa 02F02*	AJ870441	F-CTTIGCAGCTGAAGAACCTCTGA R-CAGCAGCTGCCTTAGTCCTAGT	(AGG) ₃ (AGA) ₅	61	199	+/+	+/-	+	+/-
UFFa 02F07	CO816783	F-GAGGCTTACCGTTCCAATCTTC R-GTTGGGATCCTCTAACATCTGG	(CT) ₁₅	50	182	+/+	+/-	+	+/+
UFFa 02G01	CO816786	F-ACGAGGTGGTTTTGTGTGT R-CCCAGATGAAGAACCGATCTA	(AG) ₆	64	159	+/+	+/-	+	+/-
UFFa 02H04*	AJ870442	F-ATCAGTCATCCTGCTAGGCACT R-TACTCTGGAACACGCAAGAGAA	(TCG) ₆	63	202	+/+	+/-	+	+/-
UFFa 03B05*	AJ870443	F-GGAATCCAAGTACAGGCTTCA R-AAGGAGCCTCTCCAATAGCTTC	(AGC) ₆ , (CAG) ₅ (CAA) ₄	65	231	+/+	+/-	+	+/-
UFFa 03C04	CO816825	F-CGGTCAGCAGGAGATAAACAC R-GCCCCATACTACCATTATGACC	(GGA) ₅	56	239	+/+	+/-	+	+/+
UFFa 03D11	CO816840	F-GCCTTGATGTCCTGTTGAGTAG R-TACCTTCTGCATTACCATGAC	(AGA) ₅	64	189	+/+	+/-	+	+/+
UFFa 04E12	CO816927	F-GACTACCCACGGCAACAGATA R-AGGGAAAGTAGCTGGAAACCAT	(TC) ₅ , (GA) ₆	54	178	+/+	+/-	+	+/+
UFFa 04G04*	AJ870445	F-ACGAGGCCTGTCCTTGTGTA R-GCTCCAGCTTTATTGTCTTGCT	(TTC) ₇	65	187	+/-	+/+	+	+/+
UFFa 08C11*	AJ870446	F-GGACGTCCCCCTCTTATTTCT R-ACCCCACATTCCATACCACTAC	(TGG) ₆	62	203	+/-	+/-	+	+/+
UFFa 08H09	CO817234	F-CTTCACCTAACACTTGCTGA R-GGTCTGTTCCCTTCCTGTTTG	(AT) ₄ , (TC) ₄ , (TA) ₇ (TA) ₁₀	65	188	+/+	-/-	+	+/-
UFFa 09B11*	AJ870448	F-CTTGGGAGAGAACAGAAAAAC R-TCAGAACCAACTCCAGAGAAGC	(AG) ₆	65	197	+/-	+/-	+	+/-
UFFa 09E12*	AJ870449	F-CGAGGAAGTAACCTCACAGAAA R-GGTGATGGAGAGTGCTGTTAGA	(AC) ₆	64	193	+/-	+/-	+	+/-
UFFa 10H04	CO817377	F-AGATCATCAGGACAGCTACGACT R-CCTTCACAAGATAGTAACCACAGC	(GA) ₆	50	186	+/+	+/-	+	+/+

Table 1 *Continued*

Locus	GenBank Accession no.	Primer sequences	Motif	T_a	Expected size	Amplification and polymorphism			
						<i>F. xananassa</i>	<i>F. vesca</i>	<i>F. chiloensis</i>	<i>F. virginiana</i>
UFFa 10D08	CO817345	F-ACGAGGTAGCTTCTCTCATC R-CTCATAAAGCGTATCAGGAGGTC	(CT) ₁₃	58	228	+/-	+/-	+	+/-
UFFa 11G07	CO817443	F-TCTCTGTCTTCTCCGAAACT R-CTACTGCTCCAACCTCAAATCG	(AT) ₈	52	174	+/+	-	+	+/-
UFFa 11F04	CO817431	F-ACGAGGCTTGTACTCTCTCAG R-GGTTCTCTCTCTGCTTAGTG	(TC) ₇	58	133	+/+	+/-	+	+/-
UFFa 11A11	CO817389	F-ACGAGGCTCCAATAGAGTCTG R-CTGAGCAGAACGCCATAGTATCAC	(TC) ₁₁	50	165	+/+	+/-	+	+/-
UFFa 12H10	CO817535	F-GAAAATCCTCACTCTTGCTC R-AGCTCTCAATCTTCACCACAAAC	(CCT) ₈	62	282	+/+	+/-	+	+/-
UFFa 12E11	CO817505	F-CCTCCCTATTGAGGTCTATGGT R-TCACACAGTACCATCCCACATATC	(CT) ₆	50	298	+/+	+/-	+	+/-
UFFa 13C07	CO817563	F-GGAGTCAACAGTAGTGCAGGTAA R-GGTTTTCTGCAGTTGGAGTAG	(CAG) ₆	60	172	+/+	-	+	+/-
UFFa 14H09	CO817691	F-AGGCTTCCTACTCTCCCATATC R-CCAAAGCCATAGCAGACTGTAG	(CT) ₅ , CTS	64	173	+/+	+/-	+	+/-
UFFa 14F08	CO817671	F-GTTTCTCAGGGCCAAAT R-CTTGAGTAGTCTCTCACCATTG	(TC) ₉ , (TA) ₅	56	137	+/+	+/-	+	+/-
UFFa 14A11	CO817622	F-ATGAAAGAAGTAGCCACTGAGC R-TACGAGAGATACTAGCGTGCTA	(GAG) ₅	60	109	+/-	-	+	+/-
UFFa 15H09*	AJ870452	F-TTAGTAGTACGCTGCCACAAGG R-CGGCTTATCTGTAGAGCTCAA	(CAGAG) ₆	64	228	+/+	+/-	+	+/-
UFFa 16H07*	AJ870453	F-CTCTACCACCATTCAAACCTC R-CACTGGAGACATCTAGCTAAAC	(CT) ₁₁	64	248	+/+	+/-	+	+/-
UFFa 16H11	CO817855	F-GACAAGTCATCTACCTACCCAAG R-GATATTGTTGGTCAGAGACCTGAG	(TC) ₆ , (TC) ₃	56	236	+/+	+/-	+	+/-
UFFa 18H04	CO818002	F-CCTTCGTTACTCTAGTAGCTCCA R-GTGATGAAGACGATGATGAGGT	(CT) ₁₄	60	157	+/+	-	+	+/-
UFFa 19B10*	CO818022	F-ATTTCTGTGTCTCCCTCTTC R-GCTCGATCTCTAGCTTCTCTCT	(CT) ₁₀ (TC) ₆	60	183	+/+	+/-	+	+/-
UFFa 20D02	CO818118	F-CTCCATCTCCACAAATCCTCTC R-GGCTAGAGTGCATGAGATGTAGT	(TTC) ₂ , C ₃₆ (TTC) ₅	62	102	+ -	+/-	+	+/-
UFFa 20H10	CO818160	F-GATGTGCTAGGACTCATACTTGG R-TAAAAGACGAGGCCATCTGA	(AT) ₇	60	227	+/+	+/-	+	+/-
UFFa 20G06*	AJ870457	F-ACTCAACCACACATTTCACAC R-GAGAAGTTGTCAATAGTCCAGGTG	(CT) ₁₁	64	154	+/+	+/-	+	+/-

*Indicates loci that were mapped in an interspecific F_2 population derived from a cross between *Fragaria vesca* 815 and *Fragaria nubicola* 601 (Sargent *et al.* 2005).

Table 2 Number of alleles (PCR products) and range in size in bp produced by 11 EST-SSR primer pairs from a group of 70 octoploid *Fragaria ×ananassa* strawberry cultivars

Locus	No. of apparent alleles	Size range (bp)
UFFa 01D03	16	160–195
UFFa 01H05	14	231–272
UFFa 02C07	32	136–202
UFFa 02F07	17	162–197
UFFa 02G01	19	142–207
UFFa 02H04	5	196–211
UFFa 03B05	14	194–237
UFFa 03D11	15	178–215
UFFa 04G04	13	169–200
UFFa 08C11	19	195–343
UFFa 08H09	13	256–229
Average	16.1	

pair was determined by gradient PCR from 50 to 65 °C using 'Strawberry Festival' DNA. The PCR protocol consisted of one cycle of initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 93 °C for 40 s, annealing at optimum T_a for 40 s, and extension at 72 °C for 40 s. A final extension cycle at 72 °C for 30 min followed. DNA was amplified in an Eppendorf Gradient thermocycler (Brinkmann Instruments) or an MJ Research Tetrad thermocycler (MJ Research). The PCR products were separated in 3% agarose gels and scored for amplification and polymorphism after visualization by ethidium bromide staining (Table 1). The number of apparent alleles and ranges in size of 11 EST-SSRs were determined in a group of 70 cultivars of cultivated *F. ×ananassa* using fluorescently labelled forward primers (Table 2) after separating the PCR products by capillary electrophoresis using the ABI 3730 DNA Analyser (Applied Biosystems). The size standard used during electrophoresis was GENESCAN 500HD [LIZ]. Sizing data were analysed using the GENEMAPPER software (Applied Biosystems).

Thirty-seven of the 46 primer pairs (80%) amplified a PCR product in six *F. ×ananassa* genotypes (Table 1). In the 'Strawberry Festival' genome, these primers were designed to amplify one pentanucleotide-, 13 trinucleotide-, and 23 dinucleotide-containing repeat motifs. Polymorphism ranged from 3% in *F. vesca* to 78.4% in *F. ×ananassa*. In *F. virginiana*, polymorphism was high at 67.6%. The AG/CT repeat motif was the most common and was present in 82.6% of dinucleotide-containing motifs while the AAG/TTC motif

was the most frequent trinucleotide repeat (38%). The number of apparent alleles (defined as PCR product) of 13 EST-SSRs in 70 strawberry cultivars ranged from five to 32 per primer pair, averaging 16.1 (Table 2). Tests for linkage disequilibrium and for Hardy-Weinberg equilibrium could not be conducted because the studied taxa are octoploid. A BLASTN search on 21 November 2005, of the 37 SSR-containing sequences against 145 existing strawberry microsatellite sequences detected identity only to the original sequences.

Cross-species transference was high in the *Fragaria* genus and ranged from 89% in *F. vesca* to 100% in the clones representing the two octoploid strawberry progenitors, *F. chiloensis* and *F. virginiana*. Fourteen of these 46 EST-SSR loci were mapped in an interspecific *F₂* population derived from a cross between *F. vesca* 815 and *Fragaria nubicola* (Hook. f.) Lindl. ex Lacaita 601 (Table 1) (Sargent *et al.* 2005).

We expect that these unique EST-SSR markers will be useful for many molecular studies in strawberry including cultivar identification, diversity assessments, linkage mapping, and ecological and comparative studies in *Fragaria*.

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